

EXHIBIT R

survival of patients with HPV-positive SCC in comparison to those with HPV-negative SCC.

OFP-12-010

Evaluation of probe-based Confocal Laser Endomicroscopy during endoscopic resection of T1-T2 Squamous Cell Carcinoma (SCC) of head and neck: A histo-morphological correlative study
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Objective: Probe-based Confocal Laser Endomicroscopy (pCLE) is a non invasive technology which allows the achievement of "optical biopsies" and an "on line" imaging of tissues at the microscopic level. There is a major interest to evaluate this new technology during endoscopic resection of squamous cell carcinoma (SCC) of Head and Neck (HN).

Method: In a preliminary "ex vivo" study, we have demonstrated the high sensitivity and specificity of pCLE for HN lesions. We now perform a clinical "in vivo" study, in which 14/40 patients with oral or laryngeal small SCC (T1 and T2) have already been included. After topical application of Patent Blue V, images of the tumor and margins are recorded with pCLE during endoscopic resection. The reviewing of pCLE images in a separate and random way by two pathologists is a posteriori achieved and a correlative statistical study will be performed.

Results: The pathologists involved in this study are right now able to discriminate squamous cell carcinoma from non-pathological tissues on pCLE "in vivo" images of these 14 first patients.

Conclusion: This study will assess if pCLE can be routinely used in HN endoscopic resections. This new technique could be further evaluated for other indications, including robotic surgery.

OFP-12-011

Human papilloma virus reduces the prognostic value of UICC's TNM classification in tonsillar Squamous Cell Carcinomas (SCC)
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Objective: This study investigates the influence of HPV-presence on prognostic value of the UICC's TNM-classification in tonsillar squamous cell carcinomas (TSCCs).

Method: Three hundred sixty-eight TSCCs were retrospectively analysed. HPV was determined by p16, PCR and/or FISH in 335 patients. Results were correlated with patient and tumor characteristics and with the prognostic value of the current TNM-classification.

Results: 32.8 % of TSCCs were HPV-positive. They had a better overall survival ($p < 0.001$) and smaller T-stages ($p = 0.037$). N-status did not differ compared to HPV-negative TSCCs. This was reflected in a great majority of stage III/IV tumours in the HPV-positive group with smaller primary tumours ($p < 0.001$). T-status was significantly associated with prognosis ($p < 0.001$). N-status correlated with prognosis only in HPV-negative TSCCs ($p = 0.001$). Correspondingly, the UICC's staging system did correlate with prognosis in HPV-negative TSCCs ($p < 0.001$). In HPV-positive tumours, there was no significant prognostic difference between tumors staged I–IVa (resp. 100 and 88.1 %).

Conclusion: The correlation between UICC's TNM classification and prognosis is only present in HPV-negative TSCCs. As HPV-prevalence is rising, a prognostic reliable classification system in this patient group is mandatory to allow adequate stratification in interpreting clinical outcome after therapy.

OFP-12-012

Expression of cancer stem cell markers CD44 and Nanog correlate with overall survival and aggressiveness in oral Squamous Cell Carcinoma

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Objective: Recently, studies indicated that cancer stem cell plays a key role in cancer development and progression. However, the role of cancer stem cell has not been well elucidated in oral squamous cell carcinoma. The aim of this study was to investigate the relationships between the expressions of stem cell markers Nanog and CD44 and clinicopathological parameters.

Method: Immunohistochemistry and qRT-PCR were employed to analyze the protein and mRNA expression levels of CD44 and Nanog in 30 OSCC tissue samples paired with their non-tumoral margin. The results were then evaluated and compared with their clinicopathological variables as age, mean age, tumor location, tumor size-pT, nodal metastasis-pN, pathological grade, lymphatic and/or perineural invasion and recurrence.

Results: Immunohistochemistry and qPCR revealed that the CD44 was overexpressed in the OSCC when compared to non-tumoral margin. Nanog was downregulated in OSCC when compared to non-tumoral margin in both, protein and mRNA levels. Additionally, Kaplan-Meier analysis revealed that CD44 expression is associated with poor patient survival ($P < 0.05$) as well as Nanog expression is associated with aggressive tumors.

Conclusion: Taken together, these data suggest that the stem cell markers CD44 and Nanog are closely related in OSCC, and the expression of CD44 and Nanog can be used as a prognostic indicator of OSCC.

Tuesday, 2 September 2014, 08.30–12.00, ICC Capital Suite Room 17
OFP-13 Joint Oral Free Paper Session IT in Pathology/Other Topics

OFP-13-001

In vivo degradation of surgical polypropylene meshes: A finding overlooked for decades

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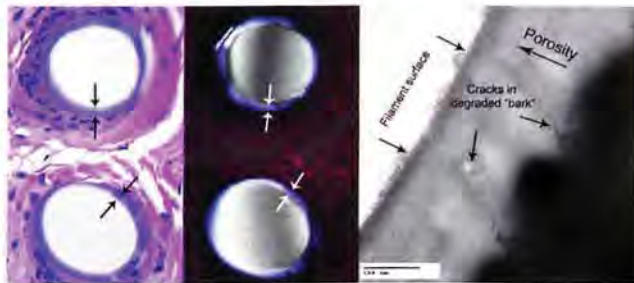
Objective: Surgical polypropylene meshes, introduced over 50 years ago are excised in up to 10 % for complications. Considering the annual worldwide use of several million devices, there is an abundant material to study the mechanisms of complications. Unfortunately, most explanted meshes are examined grossly only, while the rest is given a superficial microscopic assessment.

Method: We studied 103 explanted meshes of different designs, manufacturers and anatomical sites using conventional and transmission electron microscopy.

Results: We detected degradation of polypropylene, generally regarded as an inert material. The degraded polymer formed a demarcated layer at the surface of the filaments similarly to a tree bark. The bark traps histological dyes due to its porosity and is easily visible by conventional microscopy. A number of findings confirmed that the bark originates from polypropylene itself and forms in-vivo.

Conclusion: An easily visible by conventional microscopy, the finding has been passing unrecognized through pathologists' microscopes for decades. At the same time the manufacturers did not conduct studies of the explanted meshes. This created a paradoxical lack of knowledge in the presence of study material and readily available tools. The discovery opens the door to study the role of degradation in the development of complications.

Degradation of polypropylene in regular and polarized light and by transmission EM:



OFP-13-002

Stratifying high risk early stage colorectal cancer patients through image analysis

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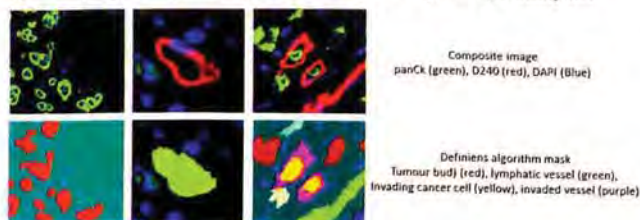
Objective: To stratify high risk early stage colorectal cancer patients through the automated quantification of three histopathological features; lymphatic vessel density and invasion as well as tumour budding. We also aim to discover novel histopathological features through Tissue Phenomics.

Method: Immunofluorescence of whole tissue sections allows visualisation of epithelial cells (pan cytokeratin), lymphatic vessels (D2-40) and nuclei (DAPI). Through Definiens image analysis of the immunofluorescently labelled tissue we quantify the three histopathological features for prognostic evaluation as well as perform unbiased multi-parametric image analysis to discover novel prognostic features through Tissue Phenomics.

Results: After image based quantification all three histopathological features were found to be predictors of poor outcome; Tumour buds (HR=5.7; 95 % CI, 2.38–13.8), lymphatic vessel density (HR=5.1; 95 % CI, 2.04–12.99) and lymphatic vessel invasion (HR=9.9; 95 % CI, 3.57–27.98). A novel image based prognostic signature was identified, through Tissue Phenomics, which allows the stratification of high risk colorectal cancer patients.

Conclusion: Utilising automated image analysis allows the robust quantification of three prognostic histopathological features in a standardised manner which reduces observer variability. Tissue Phenomics allows the identification of novel prognostic signatures from the unbiased image based quantification of the complex cancer microenvironment.

Quantifying histopathological features through image analysis:



OFP-13-003

The international collaboration on cancer reporting: Development of evidence-based core datasets for pathology cancer reporting

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Objective: To develop a process for the production, dissemination and implementation of international evidence-based pathology cancer datasets (IPCDS).

Method: The International Collaboration on Cancer Reporting (ICCR) was established in 2011 between the Pathology Colleges and Associations of the USA, UK, Canada and Australia. Cancer datasets from various organisations are harmonised and updated by internationally recognised pathologists and subjected to evidentiary and worldwide review, followed by publication in peer reviewed journals. Key international cancer organisations endorse and participate in the process.

Results: Four datasets have been published and posted to the ICCR website to date. The International Agency for Research in Cancer (IARC) has partnered with ICCR to synchronise the publication of subsequent ICCR datasets with future WHO Tumour Classification volumes. The ICCR is engaging with organisations involved in tumour staging including the Union for International Cancer Control (UICC) and the American Joint Commission on Cancer (AJCC). The European Society of Pathology (ESP) joined ICCR as a founding member in 2013, bringing over 68 countries and more than 1 billion people under a common process.

Conclusion: The ICCR has developed an efficient process for the production of standardized and evidence-based IPCDS. Engagement with key international cancer and pathology organisations will foster their adoption worldwide.

OFP-13-004

Is it safe to use digital pathology for biopsy diagnosis?

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Objective: To provide proof of concept for digital pathology in biopsy diagnosis

Method: We scanned and examined 405 cases composed of diagnostic breast biopsies (350) and histopathology surgicals (55) directly on computer screens. The diagnoses were compared to those given originally by microscopy. The five pathologists examined cases independently on hospital and home computer screens through the internet.

Results: All final diagnoses made by digital pathology on 55 general surgicals were comparable to those given by microscopy. The B category (B1–B5) of 350 diagnostic breast biopsies showed 98 % agreement. The discordance in tumour grade was 4 %. The time required for examining digitised images on computer screens was twice as long as that of microscopy with 100 MB link to hospital server. An upgrade to 1GB resulted in comparable times to microscopy.

Conclusion: The overall diagnostic agreement between digital pathology and microscopy suggests that digitised images can safely be used for diagnostic purposes. Software upgrade for better concordance in tumour grade is required. The inbuilt software including ability to measure, annotate, copy and paste images are additional advantages. The same case can be viewed by all five pathologists at the same time from different locations allowing for instant second opinion.

OFP-13-005

The effects of tissue processing on glomerular basement membrane thickness: A multicentre study

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Objective: Establishing glomerular basement membrane (GBM) thickness is important in the diagnosis of some renal diseases. In this study we aimed to determine what the influence of tissue processing schedules used in diagnostic EM centres across the UK and Eire might have on final GBM thickness.

Virchows Archiv : an international journal of
v. 465, suppl. 1 (Aug. 2014)
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2014 VOLUME 465 ISSUE 1 SUPPLEMENT

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